SEGMENTATION, AUTOTOMY AND REGENERATION OF LOST POSTERIOR SEGMENTS IN *HARMOTHOE IMBRICATA* (L.) (POLYCHAETA : POLYNOIDAE)

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ABSTRACT

In regular monthly samples from a population of *Harmothoe imbricata*, between 8 and 35% of the individuals were found to be regenerating lost posterior segments. Breakage always occurred cleanly at an intersegmental boundary and the body wall at this point is specialized for autotomy. The septa are inserted just anterior to the plane of autotomy in each segment. The septa are displaced from the transverse plane in *H. imbricata* by the insertions of two oblique muscles which have become functionally intersegmental. Neither starvation nor removal of the brain influences the rate at which posterior segments are regenerated after experimental ablation.

INTRODUCTION

In many polychaete species, parts of the body are lost to predators. The posterior body regions of errant species and the feeding tentacles of tube-dwelling species appear to be particularly vulnerable. In some species breakage occurs at a predetermined point, presumably allowing the rest of the animal to escape predation, and regeneration of the lost part normally occurs. There have, however, been few quantitative studies of the importance of such losses in natural populations or of the mechanism by which autotomy is brought about. In the present paper some preliminary observations are recorded on the processes of autotomy and regeneration in the polynoid polychaete *Harmothoe imbricata* (L.).

MATERIAL AND METHODS

Samples were collected each month from the lower littoral zone on the north side of Cullercoats Bay, Northumberland, England, between September 1968 and June 1969 (Daly 1972). Each sample contained 70-160 animals and the number which had lost and were regenerating posterior segments was recorded. The number of segments lost and the number regenerated were also noted for each of these individuals. The structure of the intersegmental regions where autotomy occurred was examined using material fixed in Bouin, double embedded in celloidin

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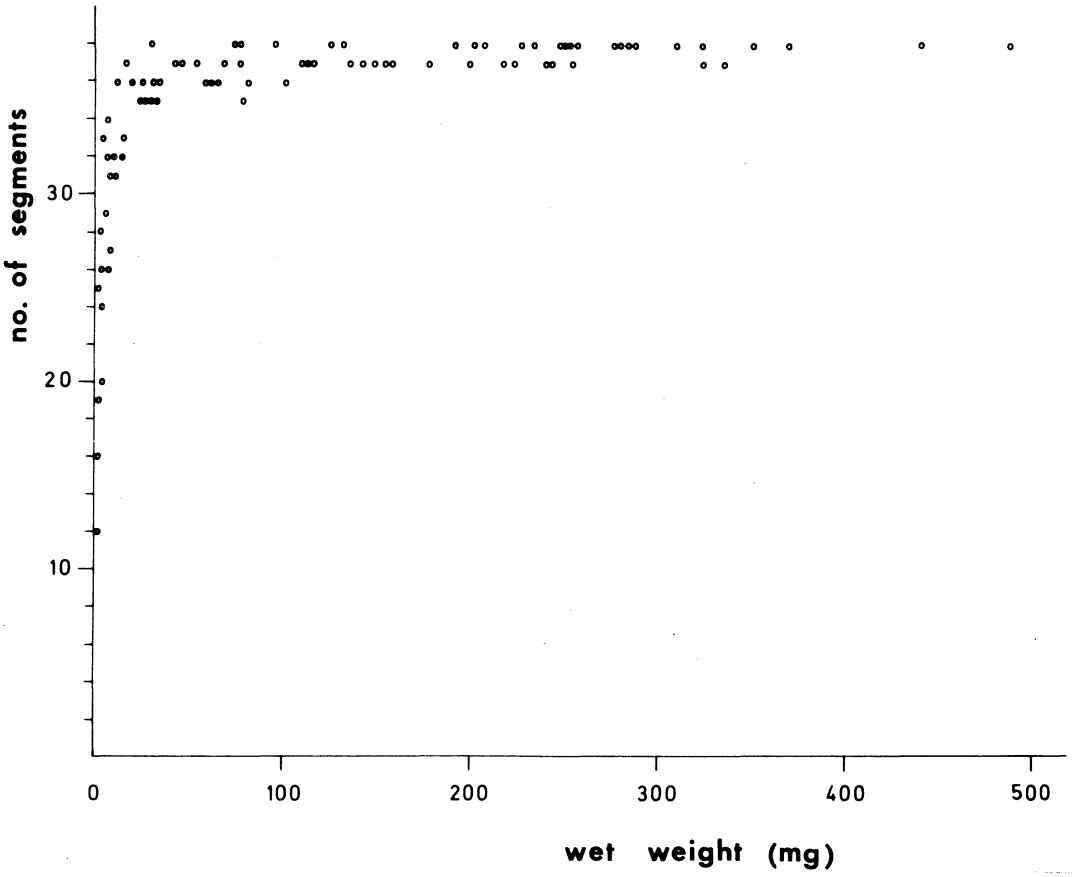

Fig. 1. The relationship between number of segments and body weight of H. imbricata. Measurements from samples collected in July and September 1968.

TABLE 1. NUMBERS OF ANIMALS REGENERATING LOST POSTERIOR SEGMENTS IN SUCCESSIVE MONTHLY SHORE SAMPLES FROM SEPTEMBER 1968 TO JUNE 1969

	Number in sample	Number regenerating	% regenerating
September	112	10	8.9
October	120	23	19.2
November	97	12	12.4
December	80	12	15.0
January	96	10	10.4
February	77	8	10.4
March	88	18	20.4
April	160	32	20.0
May	88	23	26.1
June	71	25	35.2

and paraffin wax and sectioned at 8 μm. Serial sections were cut in all three planes and were stained with Azan (Humason 1967). The arrangement of intersegmental septa was established by a study of these serial sections and by dissection of fixed material. Single segments fixed in Bouin were also stained as whole mounts using Borax Carmine (Pantin 1964). The ability of these animals to regenerate anteriorly and posteriorly was studied experimentally by cutting each of a group of 15 animals in half transversely between segments 20 and 21. The animals were first narcotized in 0.07% MS222 in sea-water. Both anterior and posterior halves were maintained individually in dishes of filtered sea-water and all fragments survived for more than 30 days.

To investigate the effect of removing the brain on posterior regeneration three groups of 13 animals each were set up in September 1969. These were checked to ensure that they were not already regenerating lost segments and animals were assigned to the three groups so that each group had an identical size frequency distribution. Each animal was narcotized and a transverse cut was made to leave the anterior part of the animal with 20 segments. At the same operation, the brain and infracerebral gland were removed from animals of the first group, the second and third groups acting as controls. Removal of the brain prevents feeding so the effect of starvation alone was tested by feeding one group of controls on *Mytilus* tissue while starving the other group. After operation, animals were placed individually in small plastic dishes containing filtered sea-water. The water was changed each day and there was no mortality. After 30 days each animal was again narcotized and the number of posterior segments regenerated was counted. The number of segments scored was the number on which parapodia or parapodial rudiments could be recognized when the worm was examined under a binocular microscope. Throughout the experiment, worms were maintained at 12-14°C.

RESULTS

In the Cullercoats population of *Harmothoe imbricata* individuals may reach 500 mg wet weight (Daly 1972). The definitive number of segments (37-38) develop usually well before the animals reach 100 mg wet weight (Fig. 1) and further growth occurs by size increase in existing segments. Once the definitive number of segments has been produced further segment proliferation occurs only after the loss of existing segments.

The number of animals collected in monthly shore samples which were regenerating lost posterior segments are shown in Table 1. These figures are probably conservative because those animals which had lost posterior segments but which showed no sign of regenerating were not counted. This avoided the possibility that damage during collection of the sample was affecting the results but probably resulted in some genuine victims of predation being ignored. The proportions of regenerating and intact animals in these samples show significant heterogeneity ($p < 0.005$, G. test, Sokal and Rohlf 1969). Since no replicate samples were obtained it is not possible to establish whether these figures indicate real changes in the population

or sampling errors. If there are changes in the population from month to month then these could result from changes in the number of attacks by predators or in the rate of regeneration. The data does at least establish that in the Cullercoats population the loss of posterior segments, presumably as a result of attacks by predators, is a common phenomenon. It represents a considerable loss of biomass production from the population by a process closer to parasitism than to predation in the sense that individuals are not killed.

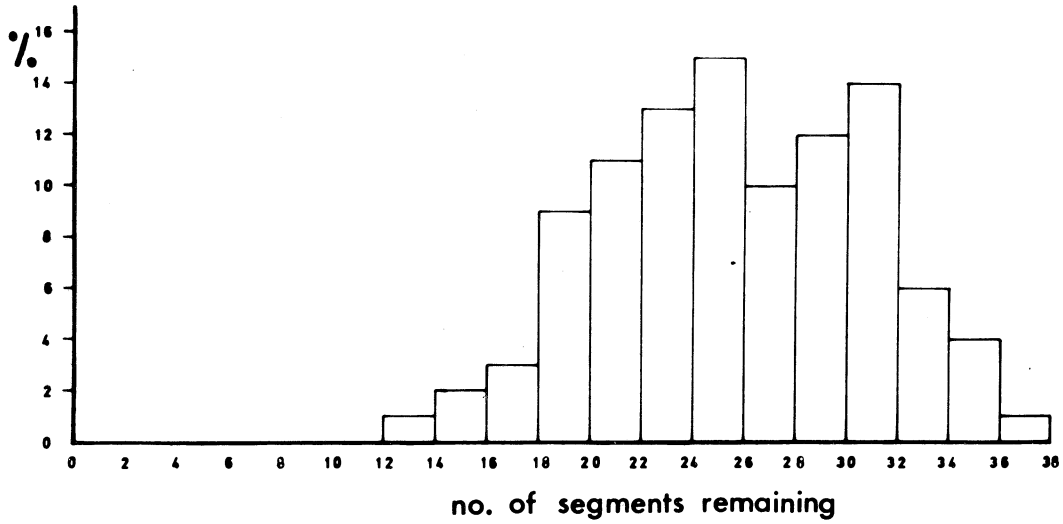

Fig. 2. Percentage frequency histogram showing the number of segments remaining for those animals which were regenerating lost segments when collected from the shore. n = 154. Animals collected between September 1968 and June 1969.

The frequency distribution of the number of posterior segments lost in those regenerating animals collected on the shore is shown in Fig. 2. From this it appears that autotomy does not occur at any specific point along the animal's body. The shape of this distribution is determined by at least two factors. Autotomy in the proboscis region, segment 1-12, would leave the proboscis projectiong from the posterior end of the fragment and this would prevent healing. Those animals, in contrast, which have lost only a very small number of segments will take less time to regenerate in the intact state so they will occur relatively less frequently in samples.

No posterior fragments regenerating a new head end were ever found on the shore. It is assumed that any such fragments left by a predator would also soon be attacked and eaten. In the laboratory posterior fragments heal over at the cut anterior surface and survive for over 30 days without showing any trace of regeneration. These fragments will swim rapidly when touched but show little spontaneous activity. In the absence of the brain, anterior sense organs and proboscis, the animals are unable to feed.

Autotomy occurs as a clean intersegmental break and the line of fracture is visible externally as a shallow groove around

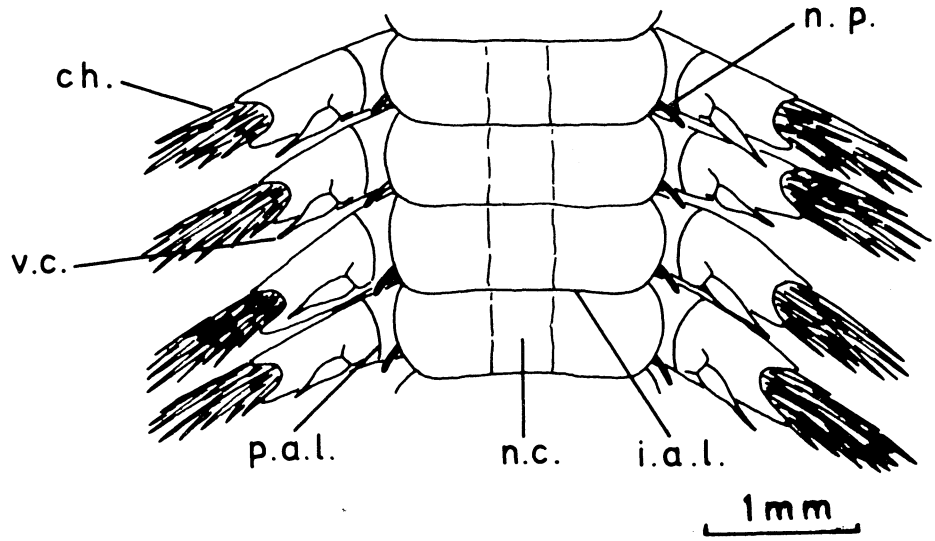

Fig. 3A. Drawing of four segments from the mid-body region of H. imbricata seen from the ventral side to show autotomy lines. ch - chaetae, i.a.l. - intersegmental autotomy line, n.c. - nerve cord, n.p. - nephridial papilla, p.a.l. - parapodial autotomy line, v.c. - ventral cirrus.

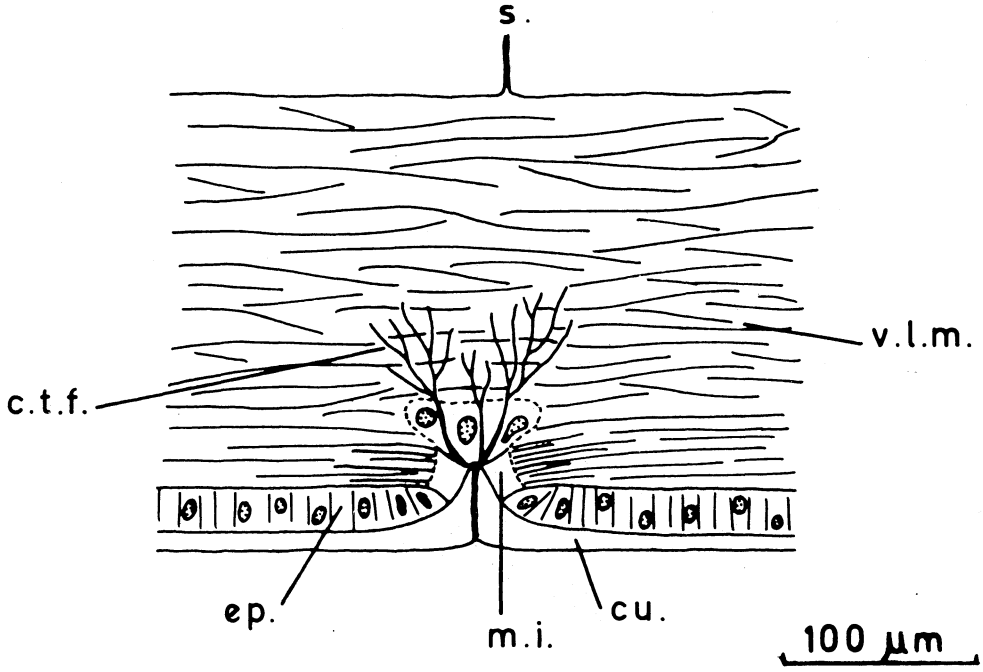

Fig. 3B. Drawing of parasagittal section through body wall of H. imbricata in the region of the ventral longitudinal muscle to show the structure of the intersegmental autotomy line. cu - cuticle, c.t.f. - connective tissue fibres, e.p. - epithelium, m.i. - muscle insertion, s - septum, v.l.m. - ventral longitudinal muscle.

the animal between successive segments. No animals in which a break had occurred in any other plane were found on the shore. the structure of the intersegmental line in another polynoid *Polynoe scolopendrina* has been described by Clark and Richardson (1967). The position of the intersegmental line and its structure as seen in sagittal section are shown in Figs 3A and B. It consists, as Clark and Richardson described, of a cuticular infolding associated with strands of connective tissue which branch among the underlying muscle fibres. In *H. imbricata*, there is a nerve which runs laterally from the ventral nerve cord in association with each intersegmental line. In the dorsal and ventral longitudinal muscles, some distal fibres in each muscle insert onto the cuticular infolding though it is not known whether these represent functionally distinct groups of fibres within the longitudinal muscles as Lawry (1971) has suggested. A similar though less developed line of cuticular infolding surrounds the base of each parapodium (Fig. 3A) and also the base of the scales, cirri and other sensory appendages. Autotomy of each of these appendages occurs along the line of cuticular infolding.

The intersegmental line of cuticular infolding is also involved in growth of the animal. Some individuals of *H. imbricata* have a black granular pigment in the epidermis. If these animals are fed on large numbers of *Scolelepis*, rapid growth occurs and associated with this is the appearance of pale transverse bands of new epithelium and cuticle both anterior and posterior to the intersegmental line.

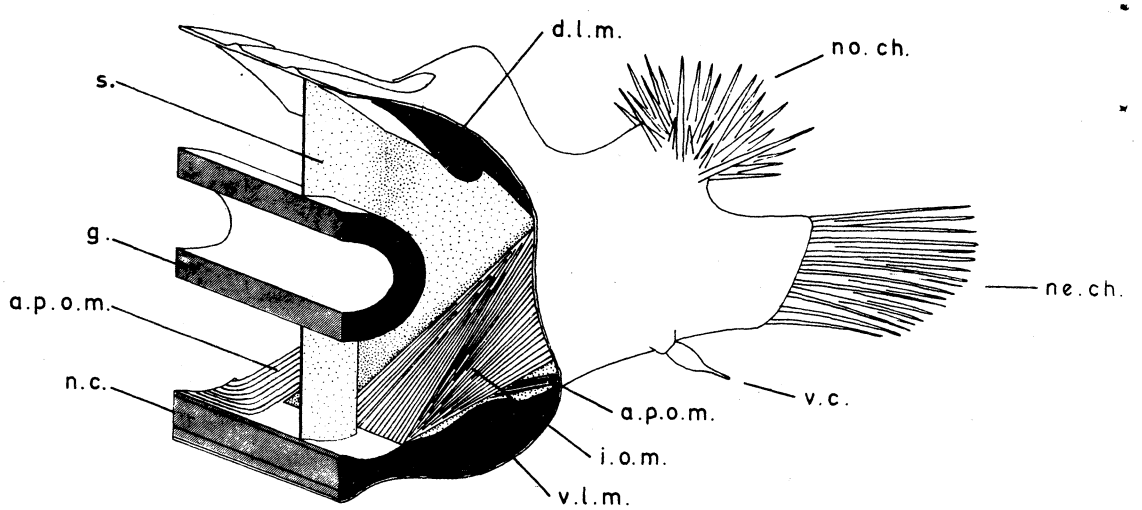

Fig. 4. **Drawing of half segment from right side of H. imbricata to show the position of the septum.**
a.p.o.m. - anterior parapodial oblique muscle, d.l.m. - dorsal longitudinal muscle, g - gut, i.o.m. - interparapodial oblique muscle, n.c. - nerve cord, ne.ch. - neuropodial chaetae, no.ch. - notopodial chaetae, s - septum, v.c. - ventral cirrus, v.l.m. - ventral longitudinal muscle.

The septa are inserted anterior to the line of cuticular infolding and effectively seal off the segment anterior to the break after autotomy. In *H. imbricata* the septa are complete but do not form simple transverse sheets of tissue in the intersegmental region as they do in many polychaete species (Clark 1962). The arrangement of a septum and the adjacent muscles is shown diagrammatically in Figs 4 and 5. The nemenclature used to describe the muscles is that developed by

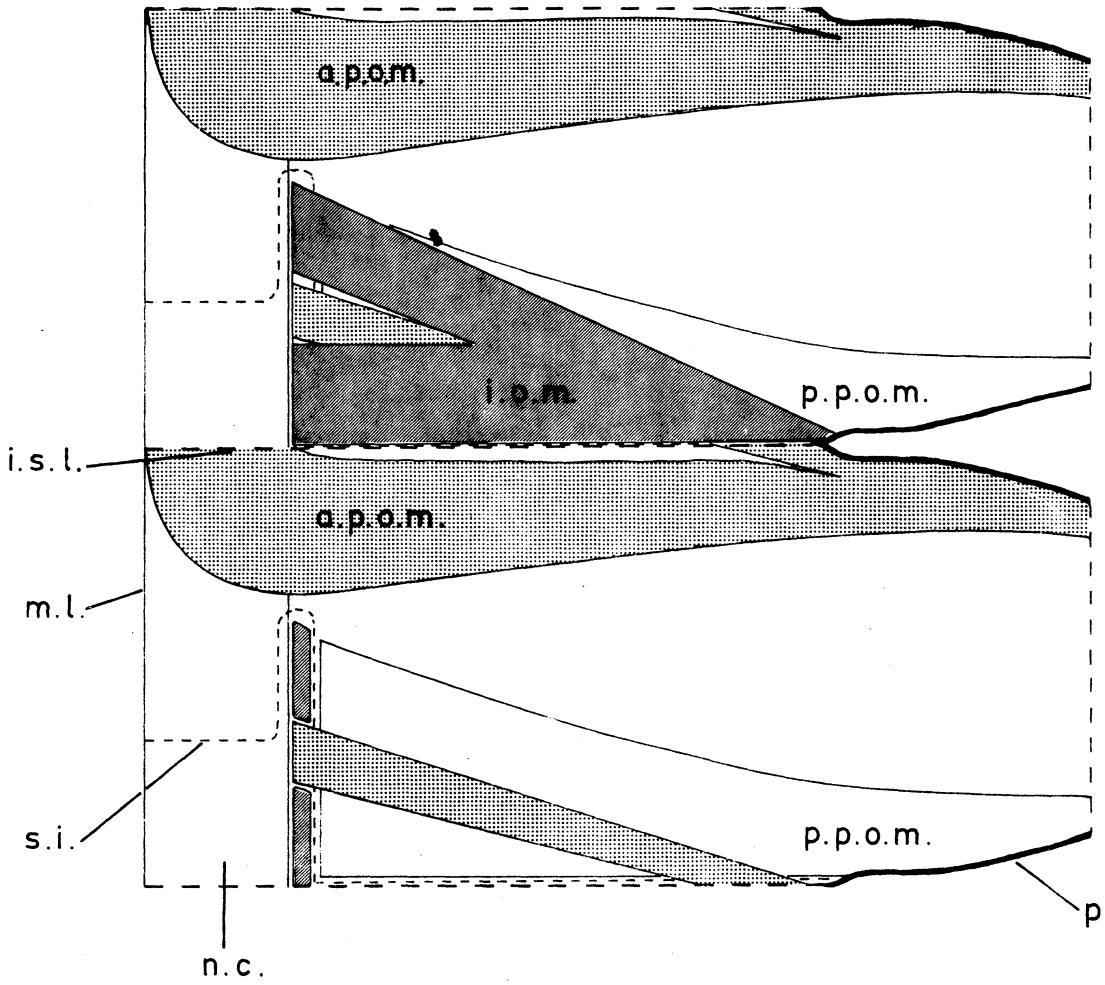

Fig. 5. Plan diagram of two half segments from right side of H. imbricata seen from above. The dorsal body wall and the gut have been removed and only the bases of the parapodia are shown. In the posterior segment the interparapodial oblique muscle has been removed to show more clearly the position of the two parapodial oblique muscles.
a.p.o.m. - anterior parapodial oblique muscle (stippled), i.o.m. - interparapodial oblique muscle (cross hatched), i.s.l. - intersegmental line (heavy dashed), m.l. - mid-line of animal, n.c. - nerve cord, p - parapodium, p.p.o.m. - posterior parapodial oblique muscle (not shaded), s.i. - septum insertion on ventral body wall.

Mettam (1967, 1971). The anatomy of the segmental muscles in *H. imbricata* has recently been described by Lawry (1971) although he did not describe the position of the septa. The displacement of each septum from the transverse plane in *H. imbricata* is probably due to the shifting of the insertion of two oblique muscles forward of the intersegmental line. These two are the large interparapodial oblique muscle and a part of the anterior parapodial oblique. These muscles are thus functionally intersegmental because they insert in two adjacent segments. The septa however have merely been displaced anteriorly rather than penetrated by these muscles. Mettam (1971) has shown that the septa in the mid-body region of *Aphrodite* may be displaced anteriorly from the intersegmental plane. He interpreted this to be the result of posterior displacement of the parapodia with reference to the septa while in *H. imbricata*, the converse situation (a displacement of the septa with reference to the external segment) seems a more likely explanation. No trace could be found of the dorso-ventral muscles figured by Lawry (1971) in *H. imbricata* although numerous specimens were examined. The septum, as can be seen from Fig. 4, follows a complicated course from its ventral insertion just anterior to the intersegmental line. It runs forward above the posterior parapodial oblique muscle but below the anterior parapodial oblique and interparapodial oblique muscles. The septum then turns posteriorly over the inter-parapodial oblique muscle before running almost vertically to its insertion on the gut, on the dorsal body wall and on the dorsal longitudinal muscle. As in *Nereis diversicolor* (Mettam 1967) the lateral insertion of the septum is anterior to the insertion of the interparapodial oblique muscles which in turn are inserted just anterior to the intersegmental line. Muscle fibres are present in the septa of *H. imbricata* although in the interests of clarity these have been omitted from the drawing in Fig. 4. The arrangement of muscle fibres is similar to that in *Lepidonotus clava* (Clark 1962) although the muscles in *H. imbricata* are much less developed than they are in *Lepidonotus*.

The blood system in *H. imbricata* is not extensive (Daly 1972) so that mechanisms to prevent blood loss after autotomy are probably not very important. It seems likely that much of the circulation in these animals involves the coelomic fluid as Segrove (1938) suggested, rather than the blood although neither carries any pigments.

The results presented in Fig. 6 show that regeneration of experimentally removed posterior segments in *H. imbricata* is not significantly affected either by starvation or by removal of the brain (in each case $p > 0.1$, Mann Whitney U test with correction for ties, Siegel 1956).

DISCUSSION

Growth in polychaetes has two components, the proliferation of new segments at the posterior end and the increase in size of existing segments. There is no evidence that individuals of any polychaete species ever reach a definitive maximum size and then stop growing. The rate of growth may slow down with

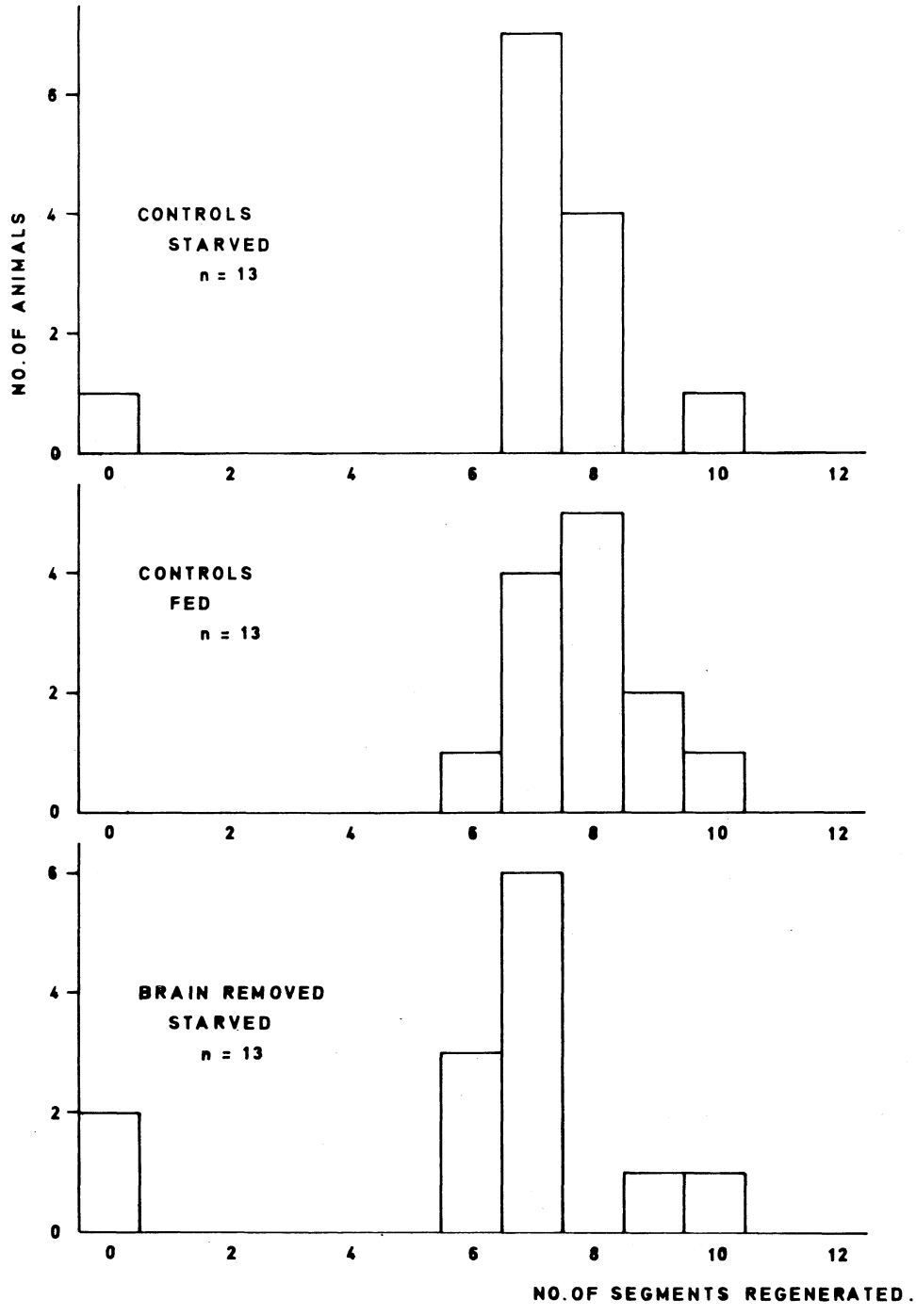

Fig. 6. The effects of starvation and of brain removal on the number of segments regenerated within 30 days of operation by H. imbricata.

age or at the onset of maturity as it does in *Nereis diversicolor*. Growth may even be reversed experimentally by starvation as in *Harmothoe imbricata* (unpublished observations). The relative contribution made by the two components to the overall growth of the animal varies widely both between species and during the life-history of individual animals. In some species, *Nereis diversicolor* (Clark and Scully 1964), *Nephtys hombergi* (Clark and Clark 1962) and *Cirratulus cirratus* (Olive 1970), both segment proliferation and segment growth occur throughout the life of the individual. In other species, *Clymenella torquata* (Moment 1951), and *Harmothoe imbricata*, the individuals proliferate a definitive number of segments and further growth occurs solely by size increase in existing segments.

The ability to regenerate lost posterior segments appears to be a general phenomenon amongst the polychaete species which have been examined so far. Clark and Clark (1962) state that the short bodied polynoids have no power of regeneration. This is certainly not true for *H. imbricata*, and several other polynoid species (*Harmothoe impar, H. extenuata, Lepidonotus squamatus* and *Halosydna gelatinosa*) show a comparable ability to regenerate (unpublished observations). The ability to regenerate anterior segments, however, is present in some polychaetes but not in others. Thus *Procerastea halleziana* (Allen 1921), *Dodecaceria caulleryi* (Dehorne 1933), *Clymenella torquata* (Moment 1951) and *Pygospio elegans* (Rasmussen 1953) are all able to regenerate anteriorly. *Chaetopterus variopedatus* is able to regenerate anteriorly providing that the cut is made anterior to segment 14 (Berrill 1928). *H. imbricata, Nereis diversicolor* and *Nephtys hombergi* in contrast show no ability to regenerate lost anterior segments at all.

Bliss (1960) in her review of autotomy mechanisms in Crustacea has defined autotomy as a breakage requiring reflex action of the animal's own musculature. In contrast she defined autospasy as breakage at a defined point of weakness by the action of some externally applied force. Thus autotomy in a crab's leg does not occur at the weakest point but at a specific point where the animal's own muscle system can overcome the fracture resistance of the cuticle. It is not clear which of these two exactly defined terms applies in the case of *H. imbricata*. Although the mechanism of breakage is not known it is likely to be autotomy because it can occur in response to the presence of noxious chemicals in the water, when no external force is applied to cause the fracture. In the case of the scales, breakage must occur by autotomy because scales are shed even from segments which are not touched when attempts are made to pick up a worm using forceps. As Nicol (1953) showed, these scales are luminous and continue to flash for some time after autotomy.

The intersegmental cuticular infoldings were described by Clark and Richardson (1967) as apodemes, providing regular insertions for longitudinal muscle fibres. They assumed that the structures increased the locomotory efficiency of the longitudinal muscles but they noted that the structures were best developed in long, thin, fragile commensal species rather

than in those polynoid species which show the best developed powers of locomotion. It thus seems more likely that these structures are primarily autotomy planes at which the animal can break if the posterior end is grasped by a large predator. Lawry (1971) has described the division of dorsal and ventral longitudinal muscles into two components, one of which is inserted on the autotomy planes while the more proximal part of each muscle runs continuously over the length of the body. It is possible that abrupt shortening of those longitudinal muscle fibres which are attached to the autotomy plane in a particular segment may be the mechanism by which autotomy occurs.

The results obtained for posterior segment regeneration in *H. imbricata* when the brain has been removed are in marked contrast to the results of parallel experiments with *Nereis diversicolor* (Golding 1967). In *Nereis diversicolor* starvation halved the rate of segment regeneration while brain removal effectively prevented any regeneration of posterior segments. The normal regeneration of lost posterior segments in *H. imbricata* is not dependent on the presence of a hormone secreted by the brain, as it is in *Nereis*. This essential role of the brain in *Nereis* may be exceptional among polychaetes. Berrill (1928) has shown that fragments of *Chaetopterus variopedatus* posterior to segment 14 are able to regenerate numerous segments posteriorly but never show any anterior regeneration. This implies that in *Chaetopterus*, as well as in *Harmothoe*, posterior segment proliferation can occur in the absence of the brain. Several polychaete species, *Procerastea halleziana* (Allen 1921), *Dodecaceria caulleryi* (Dehorne 1933), *Clymenella torquata* (Moment 1951), *Pygospio elegans* (Rasmussen 1953) are able to regenerate completely from a single segment or from small numbers of segments. These observations again imply that, in some species at least the earlier stages of posterior regeneration occur in the absence of the brain.

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